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March 10, 2008

Primary Examiner Dr. Lori A. Clow
Art Unit: 1631, Technical Center 1600,
Commissioner for Patents, USPTO,
P.O. Box 1450, Alexandria VA 22313-1450

Re: Application No.: 10/810,296 Filed Date: March 27, 2004

Enclosed Relevant Document for the Application

Dear Dr. Lori Clow,

Please find the enclosed abstract of a review article published in Nature, 21 February 2008, Vol 451, 904-913, which is related to the US patent application (application No.: 10/810,296) entitled "Multiparameter Method of Screening for Atherosclerosis-Related Coronary Heart Disease or Stroke".

The latest review article in atherosclerosis research emphasizes that certain lipoproteins such as the low-density lipoprotein (LDL) and renin-angiotensin-aldosterone system, one of inflammation pathways, or C-reactive protein (CRP), one of inflammation markers, are important in the pathogenesis of atherosclerotic cardiovascular disease (ACD). In addition, efforts to understand how risk factors such as high blood pressure, elevated LDL and CRP levels in human blood contribute to atherosclerotic diseases, are leading to new targets for therapy, as discussed in the abstract.

The US patent application is greatly supported by the latest review article because the contributions of elevated LDL and CRP levels in human blood, two main risk factors, to ACD have been integrated into the method of this invention by means of the equations (1)

and (3) in pages 3-4 of specification or in pages 9-10 of claim of the application, and this method can be not only quantitatively to evaluate the contributions of various risk factors such as the high blood pressure, elevated LDL and CRP levels to ACD and the effects of various amounts of a risk factor to the disease but combine the contributions and effects, as indicated in examples 1-5 in pages 7-8 of the specification, which is significant improvements on available screening or diagnosing methods. This invention has provided some answers to fundamental questions in atherosclerosis, for example, how to understand and evaluate the contributions of risk factors to atherosclerotic diseases.

The applicant respectfully requests that a timely Notice of Allowance is issued in this case.

Thank you for your consideration.

Sincerely,

Xing F. Wang, Ph.D.
Applicant

Encl.: the abstract of review article (1 sheet).

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Translating molecular discoveries into new therapies for atherosclerosis

Daniel J. Rader[1] & Alan Daugherty[2]

Atherosclerosis is characterized by the thickening of the arterial wall and is the primary cause of coronary artery disease and cerebrovascular disease, two of the most common causes of illness and death worldwide. Clinical trials have confirmed that certain lipoproteins and the renin–angiotensin–aldosterone system are important in the pathogenesis of atherosclerotic cardiovascular disease, and that interventions targeted towards these are beneficial. Furthermore, efforts to understand how risk factors such as high blood pressure, dysregulated blood lipids and diabetes contribute to atherosclerotic disease, as well as to understand the molecular pathogenesis of atherosclerotic plaques, are leading to new targets for therapy.

During atherosclerosis, the arterial wall gradually thickens to form an atherosclerotic plaque, resulting in the narrowing of the lumen of the artery. Consequently, the amount of blood supplied to the organ is reduced, most commonly affecting the heart and the brain. Plaques can abruptly rupture, causing a blood clot and often myocardial infarction (heart attack) or stroke. Intensive study of the cellular and molecular mechanisms that underlie atherogenesis (that is, the formation of atherosclerotic plaques) and plaque rupture has led to a consensus view of these processes[1] (Fig. 1). Initiation and progression of the lesion are highly complex processes, and many aspects of atherogenesis remain incompletely understood. Furthermore, in most cases, mechanistic insights have yet to be translated into therapeutic approaches. In this review, we discuss the most exciting advances in atherosclerosis research since 2000, emphasizing new findings that have translational and therapeutic implications. For a review of earlier findings, see ref. 2. At present, the two main conceptual approaches to therapy for atherosclerosis are manipulation of plasma lipoprotein metabolism or cellular cholesterol metabolism, and manipulation of inflammatory processes. Here we discuss both approaches, focusing on how recent findings might lead to new types of therapy. We set the scene with a discussion of how new therapeutic targets are identified and validated and then finish by looking at how genome-wide association studies are rapidly altering the way in which atherosclerosis is understood and might be treated.

Identification of therapeutic targets in humans and mice

Perhaps the most convincing evidence for a potential therapeutic target is provided when a human genetic condition arising from simple mendelian genetics is found to be associated with altered risk of atherosclerotic disease. An example is homozygous familial hypercholesterolaemia, which is caused by mutations in the gene encoding the low-density lipoprotein (LDL) receptor. The observation that this disease is associated with markedly premature atherosclerosis led to an understanding that increased concentrations of LDL cholesterol in plasma can cause atherosclerosis. This observation also led to the general concept that intervening to increase LDL-receptor expression would reduce LDL concentrations and thus the risk of atherosclerosis. However, classic mendelian disorders are not associated with most genes of interest, and even when they are, the prevalence of these disorders is usually too low to provide strong evidence of an association with atherosclerosis. By examining extended families, linkage studies have identified loci that seem to be important determinants of premature coronary artery disease, but it has often been challenging to identify the specific genes that cause disease. One notable recent success was the identification of a mutation in the gene encoding LDL-receptor-related protein 6 (LRP6) in a large family as responsible for autosomal dominant premature coronary artery disease accompanied by features of the metabolic syndrome (which is a group of risk factors that are commonly associated with coronary artery disease, including hyperlipidaemia, hypertension and insulin resistance)[3]. 'Candidate genes' are frequently tested by genotyping single-nucleotide polymorphisms (SNPs) in large cohorts (or groups) of patients and examining whether particular SNPs are associated with atherosclerotic disease. Unfortunately, many of the published association studies have not been subjected to rigorous replication[4]. Most recently, genome-wide association studies have been used in an attempt to identify genes that are significantly associated with atherosclerotic disease and its risk factors (discussed later).

Studies of genetically modified mice are also commonly used to identify and validate potential therapeutic targets, as well as to investigate atherosclerotic disease mechanisms in detail. The bidirectional flow of information between mouse and human studies has been crucial for furthering knowledge of atherosclerosis, as well as for validating new therapeutic targets. However, the relevance of mouse studies for understanding the pathophysiology of atherosclerosis in humans needs to be carefully considered. There are important differences between mice and humans with respect to two of the main processes involved in atherogenesis: lipoprotein metabolism and inflammatory pathways. In addition, there are many inconsistencies between the various studies of atherosclerosis in mice, and the basis of these discrepancies is often unclear. Strain differences might, in part, be responsible; indeed, there can be substantial genetic variation between control and experimental mice even after extensive backcrossing of both into the same strain. A lack of standardization in measuring lesion size in mice might also contribute to these discrepancies. Furthermore, there is an increasing recognition that lesion composition, rather than size, determines the acute complications of atherosclerotic disease in humans. However, compositional analysis of lesions in mice is not routine or standardized, and the implications of differing lesion composition for disease

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